Long-term trends in leaf level gas exchange mirror tree-ring derived intrinsic water-use efficiency of *Pinus cembra* at treeline during the last century

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ABSTRACT

The ability of treeline conifers in the Central European Alps to cope with recent climate warming and increasing CO_2 concentration is still poorly understood. We determined basal area increment (BAI) and tree ring stable carbon isotope ratios ($\delta^{13}C$) of *Pinus cembra* trees from 1925 through 2013. Stable isotope ratios and BAI were compared with leaf level gas exchange measurements carried out *in situ* between 1934 and 2012, and thus, provided new insights into long-term trends of tree-ring derived intrinsic water-use efficiency (iWUE). Mature *P. cembra* trees at treeline responded to increasing C_a and air temperature with a parallel increase in maximum net CO_2 uptake rate at ambient CO_2 (A_{max}) and tree-ring-derived intercellular CO_2 partial pressure (C_i). A_{max} tripled and was positively correlated to BAI and C_i. The latter increased in parallel with ambient CO_2 concentration and stomatal conductance. In contrast to the instantaneous gas exchange parameters, $\delta^{13}C$ derived iWUE informs about the long-term changes in the carbon water relations. These data showed three changes in the iWUE chronosequences, which could be identified with different long term gas exchange patterns: (1) from stomatal controlled functioning from 1925 to 1981, to a situation where (2) both net CO_2 fixation (A) and leaf conductance for water vapour (g_w), responded to the environment from 1982 to 1997, and (3) back to a stomata controlled pattern over iWUE from 1998 onwards. This temporal pattern was also mirrored in leaf level gas exchange assessments, suggesting a parallel increase of A and g_w of *P. cembra* at treeline during the last nine decades.

1. Introduction

People have raised concern about treeline associated forest ecosystems, as they may undergo significant alterations due to increasing atmospheric CO_2 concentrations and climate warming (Holtmeier and Broll, 2007; Wieser et al., 2009). Warming experiments and free-air CO_2 enrichment carried out at treeline in the Austrian and Swiss Alps indicate that ecosystem warming (Wieser et al., 2015) and increasing atmospheric CO_2 concentrations (Dawes et al., 2013; Streit et al., 2014) increased net CO_2 uptake rate of pine and larch. Transpiration (E) and leaf conductance to water vapour (g_w) were insensitive to changes in CO_2 concentration (Streit et al., 2014), while ecosystem warming increased g_w and hence also E in *Pinus cembra* at treeline (Wieser et al., 2015) in the Swiss and Austrian Alps, respectively, and in conifers in boreal forest ecosystems (Bergh and Linder, 1999; Kellomäki and Wang,

1998; Marchin et al., 2016; Van Herk et al., 2011).

At time scales of decades and longer, environmental parameters (temperature, air humidity, water availability, and ambient CO_2) also influence net CO_2 uptake rate, which is reflected in the carbon isotope ratio ($\delta13C$) of wood in tree rings (Churakova et al., 2016). $\delta13C$ in plant organic matter is related to the ratio between net CO_2 fixation (A) versus leaf conductance for water vapour (g_w), which is defined as the intrinsic water-use efficiency (iWUE = A/g_w), independent of VPD. Therefore, iWUE is not identical to the instantaneous WUE of photosynthesis (pWUE = A/E), which is the ratio A to E. In addition to g_w, pWUE depends also on evaporative demand, as E is determined by the product of g_w and leaf to air vapour pressure deficit (LAVPD = $e_i - e_a$; where e_i and e_a stand for the intercellular and the ambient vapour pressure respectively), or more precisely Δw, i.e. pressure corrected LAVPD. In contrast iWUE does not consider the evaporative demand

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but only accounts for the internal (C_i) versus the ambient (C_a) CO_2 concentration ratio (C_i/C_a). Furthermore iWUE is an integrative measure representing the carbon water relation for the whole vegetation period, including periods with high and very low assimilation rates, at low light, low temperature and dry conditions. This distinction is important, because the two parameters cover different time scales: iWUE (A/g_w) can be traced back for as many tree rings as analyzed (centuries), while pWUE (A/E), yields instantaneous information (Cernusak et al., 2013; and further references therein). This discrepancy between the isotope (Saurer et al., 2014) and instantaneous gas exchange approach is a clear result of the different time scales, as the isotopic iWUE integrates low light, low temperature and dry conditions over the whole vegetation period, whereas gas exchange derived pWUE predominantly reflects optimal conditions (near light saturation, temperatures in the range of the plants optimum photosynthetic rate and usually sufficient water supply).

The aim of this paper is to compare long-term trends in leaf-level gas exchange with tree-ring width, stable carbon isotope ratios ($\delta^{13}C$) extracted from tree-rings, and $\delta^{13}C$ derived iWUE of *P. cembra* trees growing at the treeline in the Central Tyrolean Alps, and its response to environmental changes over the past 89 years (1925–2013). As precipitation during the growing season is abundant and occurs every third to fourth day on average in this region (Wieser, 2012) soil water availability can be ruled out as a limiting factor (Matyssek et al., 2009; Mayr, 2007; Tranquillini 1979), thereby leaving other environmental influences such as temperature to be the predominant control for tree growth (Oberhuber et al., 2008). The results are expected to contribute to an increased understanding and appreciation of the importance of instantaneous gas exchange measurements and environmental conditions for the interpretation of iWUE inferred from $\delta^{13}C$ in high mountain regions where soil water limitation is absent.

2. Material and methods

2.1. Study site and climate data

The study site is located at 2000 m a.s.l on Mt. Patscherkofel (47°12′37′ N, 11°27′07′ E), south of Innsbruck, Austria at the lower edge of the treeline ecotone. According to the World Base for Soil resources (FAO 2006), the soil at the study site is a haplic podzol, a typical soil type of the treeline ecotone in the Central Tyrolean Alps formed on gneisses and schist (Neuwinger, 1972). The water holding capacity of the top soil (to 65 cm depth) at saturation (-0.001 MPa) averages 0.60 $m^3\,m^{-3}$.

The study site is characterized by a cool subalpine climate with the possibility of frost during the whole year, and a continuous snow cover from October until May. Environmental parameters for the period 1963–2010 were obtained from a weather station nearby (Klimahaus Research Station and Alpengarten; 1950 m a.s.l., approx. distance 300 m). During this period (1963–2010) mean annual air temperature (T_{air}) was 2.4 °C, mean annual relative humidity (RH) was 77%, and the mean annual precipitation (P) was 864 mm, with the majority falling during the growing season (May throughout October). Due to ample P throughout the growing season, soil water potential seldom dropped below -0.01 MPa, ($\approx$ soil water contents above 0.35 $m^3\,m^{-3}$), including the hot and dry summer of 2003 (Wieser, 2012).

A longer climate data series for the Mt. Patscherkofel region reaching back to 1866 (Böhm et al., 2001) is available from the Austrian Zentralanstalt für Meteorologie und Geodynamik (ZAMG) climate station in Innsbruck (582 m a.s.l.) approximately 6 km north-west from the field site. Climate data from Innsbruck were highly correlated with those from our treeline site. Within the overlapping time interval (1963–2010) mean annual total P was without any significant difference (y = 0.995x; r^2 = 0.71 P < 0.001) between the treeline site (864 mm) and Innsbruck (867 mm). Mean annual T_{air} decreased with elevation (y = 0.97 x − 7.1, r^2 = 0.77, P < 0.001), according to a

temperature laps rate of 0.55 K per 100 m of elevation. Our estimated laps rate of 0.55 K per 100 m of elevation matches the mean year round laps rate of 0.55 K per 100 published for the European Alps (Baumgartner, 1980; Franz 1979). Mean annual RH was at average 8% higher at treeline (77%) as compared to the Innsbruck station (69%). Given such agreement, the existing time series (1962–2011) of P, T_{air}, and RH was extended to the early end to 1925 and at the recent end to 2013 through linear regressions with the database from the Innsbruck climate station.

In this study we focus on *P. cembra* L., which is the dominant and widespread tree species at treeline in the Central Eastern Alps and accounts for 84% of the tree population within the study area at Mt. Patscherkofel. *Larix decidua* Mill. (9%) and *Picea abies* L. Karst (7%) are scattered at some locations. The trees grow either as isolated trees or in groups of four to five.

2.2. Sampling and dendrochronological procedure

We used dendrochronological methods to assess changes in stem radial growth. In fall 2013 we sampled 10 dominant *P. cembra* trees. The distance between solitary trees and between groups is 20–30 m. In 2013 the selected study trees were 125 $\pm$ 4 years old, and their stem diameter at breast height (DBH) averaged 37 $\pm$ 3.2 cm. The stem height averaged 12 $\pm$ 1.3 m. Two sample cores per tree (S and W exposure) were extracted with a 5-mm-diameter increment borer at breast height. In the laboratory the cores were non-permanently mounted on a holder, dried for contrast enhancement of tree ring boundaries, and the surface was prepared with a razor blade (Pilcher, 1990). Using a reflecting microscope (Olympus SZ61) and the software package TSAP WIN Scientific, ring widths were measured to the nearest 1 μm. For each sample tree the ring widths of both cores were averaged, and residual chronologies were calculated using the ARSTAN software (Cook, 1987; Holmes, 1994) through estimation of the Expressed Population Signal (EPS; Wigley et al., 1984).

To overcome the problem that ring width decreases with tree maturation, ring width was converted to basal stem area increment (BAI) according to:

$$BAI = 3.14\ (R_n^2 - R_{n-1}^2) \tag{1}$$

where R is stem radius inside tree bark and n is the year of tree ring formation (Fritts, 1976). Finally BAI of each year were averaged over the ten sample trees.

2.3. Stable isotope analysis, ^{13}C discrimination, and intrinsic water-use efficiency

For carbon isotope analysis we selected these six trees out of the 10 trees used for BAI estimation, which had the strongest correlation to the site-specific tree-ring chronology, no missing rings, and regular ring boundaries. We performed the $\delta^{13}C$ analyses on the same cores as used for BAI assessment, and only sampled the most recent 89 years (1925–2013) of ring formation in order to avoid juvenile age effects on the tree ring isotope signatures (Heaton, 1999; but see McDowel et al., 2011). Annual rings (early wood plus late wood) were cut from each core at the ring boundaries using a scalpel and a reflecting microscope (Wild 308700). In *P. cembra* $\delta^{13}C$ signatures of bulk wood and cellulose have been shown to yield highly correlated signals, and on average $\delta^{13}C$ in bulk wood was lower by 1.0‰ than in cellulose (Wieser et al., 2016). Therefore, due to the ease of processing, we used bulk wood samples for our $\delta^{13}C$ analysis.

For analyzing $\delta^{13}C$, 2.0 $\pm$ 0.02 mg of homogenized samples were packed into tin capsules (3.5 × 5 mm, IVA Analysentechnik e. K., Meerbush, Germany) and combusted to CO_2 in an elemental analyzer (Eurovector EA3000) connected to an isotope ratio mass spectrometer (Isoprime, Elementar, Hanau, Germany). The isotope abundance was

expressed in the delta (δ) notation in ‰ relative to the Vienna Pee-Dee Belemnite (V-PDB) standard:

$$\delta_{sample} = (R_{sample}/R_{standard} - 1) * 1000 \qquad (2)$$

where R_{sample} and $R_{standard}$ represent the $^{13}C/^{12}C$ ratios of the sample and the V-PDB standard, respectively. The analytical precision was < 0.12‰ (expressed as standard deviation of an internal laboratory standard using identical sample mass). Tree ring specific $\delta^{13}C_{tring}$ was corrected for the progressive decline in atmospheric $\delta^{13}C_{atm}$ by calculating ^{13}C discrimination ($\Delta^{13}C$):

$$\Delta^{13}C \ (‰) = (\delta^{13}C_{atm} - \delta^{13}C_{tring})/(1 + \delta^{13}C_{tring}/1000). \qquad (3)$$

where $\delta^{13}C_{atm}$ and $\delta^{13}C_{trin}$ are the $^{13}C/^{12}C$ ratios in atmospheric CO_2 and tree rings, respectively. In order to connect carbon isotope discrimination with physiological responses $\Delta^{13}C$ can also be calculated using the simplified linear model of Farquhar et al. (1982):

$$\Delta^{13}C = a + (b - a) * C_i/C_a \qquad (4)$$

where a (= 4.4‰) refers to the lower diffusivity of $^{13}CO_2$ relative to $^{12}CO_2$ in air, b (= 27‰) is the isotopic fractionation caused by enzymatic C fixation, and C_i and C_a are the CO_2 concentrations in the intercellular space of the needles and the atmosphere, respectively. Values for C_a and $\delta^{13}C_{atm}$ were obtained from published data (McCarrol and Loader, 2004), recent measurements of C_a (http://cdiac.ornl.gov/trends/co2/sio-mlo.html) and $\delta^{13}C_{atm}$ (www.scrippsco2.ucsd.edu/home).

Intrinsic water-use efficiency (iWUE), defined as the ratio of the net carbon gain (A) to leaf conductance for water vapour (g_w), was calculated as follows (Farquhar et al., 1982):

$$iWUE = A/g_w = (1-(\Delta^{13}C-a)/(b-a))* C_a/1.6, \qquad (5)$$

where 1.6 is the ratio of the diffusivities of water vapour and CO_2 in air.

2.4. Leaf level gas exchange data

We compiled published gas exchange data obtained from mature field grown *P. cembra* trees in order to demonstrate long term trends in foliar CO_2 and H_2O gas exchange. These measurements were carried out at treeline on Mt. Patscherkofel between 1934 and 2007 and at treeline in Haggen in 2012 (Table 1). The Haggen site is located 30 km west of Innsbruck near St Sigmund in the Sellrain Valley, Tyrol, Austria at 2150 m a.s.l. (47°12′42″N, 11°05′04″E), and conditions at Haggen (Kronfuss, 1997; Neuwinger, 1972; Wieser et al., 2015) are comparable to site conditions of Mt. Patscherkofel (Wieser, 2012). Maximum net CO_2 uptake rate at ambient CO_2 (=A_{max}; *sensu* Larcher, 2001) was assessed of sun exposed twigs from the upper canopy on bright and warm days between mid-summer and early September. The first in situ studies of gas exchange at treeline were published by Cartellieri (1935) who used the titration technique developed by Boysen-Jensen, adapted for field use by Bosian (1933) to determine CO_2 uptake of *P. cembra* in situ on several days during the year 1934. His published seasonal pattern of CO_2 uptake agrees well with results obtained in greater detail

when working with detached twigs (Pisek and Winkler 1958) or in situ (Havranek, 1981; Wieser, 2004) 22–68 years later (Table 1). In situ gas exchange measurements were performed by means of thermoelectrically climate-controlled cuvettes (Siemens, Erlangen and Walz, Effeltrich, both Germany) and a portable exchange system (CIRAS 1, PP Systems, Hitchin, Hertfordshire, UK). Total needle surface area and dry weight based gas exchange data published by Cartellieri (1935) and Pisek and Winkler (1958), respectively, were pressure corrected, while all the other data were calculated according to Von Caemmmerer and Farquhar (1981) and related to total needle surface area. A specific leaf area of 66 g cm^{-2} of total needle surface area (Cartellieri, 1935; Wieser, 2004) was used to convert dry mass based A_{max} values (Havranek, 1981; Koch et al., 1968; Pisek and Winkler, 1958) into area based values. For further methodological details, see publications given in Table 1.

2.5. Data analysis and theoretical considerations

We applied least squares linear regression analyses to calculate significances of long-term trends in our observed inter-annual trends of BAI, $\delta^{13}C$, $\Delta^{13}C$, C_i, C_i/C_a, C_a-C_i, and iWUE. In order to warrant persistence on the influence of environmental factors on BAI, $\delta^{13}C$, $\Delta^{13}C$, C_i, C_i/C_a, C_a-C_i, and iWUE on the long term, we divided the analyzed period (1925–2013) into the three sub-periods periods: (1) 1925–1981, a period with no considerable trend mean annual T_{air}, (2) 1982–1997, a period in which mean annual T_{air} significantly increased, and (3) 1998–2013, yet again a period with no considerable trend mean annual T_{air}. (Fig. 1a; Table 2).

These three different periods were analyzed according to Saurer et al. (2004), who showed that trees can vary in their gas exchange patterns. Either 1) C_i remains constant or 2) the ratio C_i/C_a remains constant, 3) or difference C_a-C_i remains constant.

- In scenario 1 we would find a constant C_i, resulting in an increase of $\delta^{13}C$, while C_i/C_a and $\Delta^{13}C$ should decrease (see Eqs. (3) and (4)). On the other hand C_a-C_i and iWUE will increase. This would indicate an increase in A while g_w would remain constant.
- In Scenario 2 $\delta^{13}C$ of the plant organic matter will decrease along with a decrease in $\delta^{13}C$ of the atmospheric CO_2, because C_i/C_a and $\Delta^{13}C$ stay constant. Consequently iWUE remains constant or will slightly increase, along with C_i. This scenario represents a proportional regulation of A and g_w (Wong et al., 1979)
- In Scenario 3 C_i increases with C_a, while C_a-C_i will stay constant, yet C_i/C_a could moderately increase. Accordingly $\delta^{13}C$ will decrease, as a result of an increase in $\Delta^{13}C$ while iWUE will not improve. This scenario indicates a stomatal regulation (g_w).

More details with application to real data are provided in Saurer et al. (2004)

We also tested for significant differences in tree-ring variables in different periods by means of one way ANOVA. We calculated Pearsońs correlations to examine the impact of environmental variables on A_{max},

Table 1
Measurements of maximum net CO_2 uptake rate at ambient CO_2 (A_{max}), transpiration (E), and estimates of instantaneous water-use efficiency of photosynthesis (pWUE) in sun exposed twigs from the upper canopy of mature field grown *P. cembra* trees at treeline in the Central Tyrolean Alps between 1934 and 2012. n d = not determined.

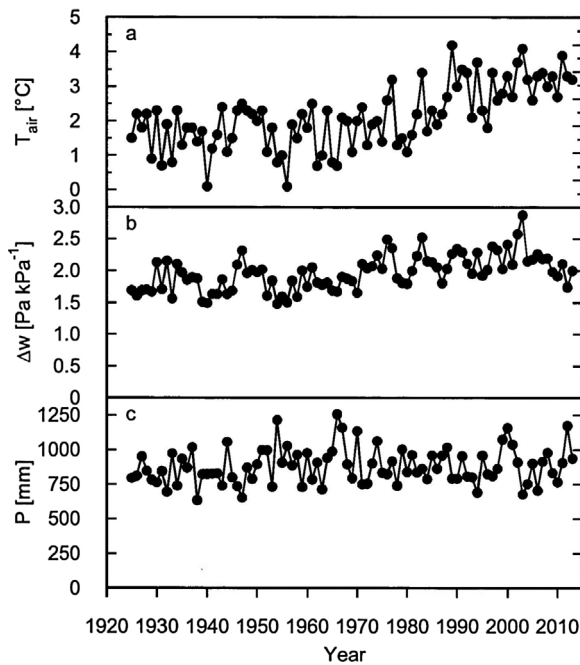| Year	Location	Measured trees	Approach	A_{max}	E	pWUE	Reference
1934	Mt. Patscherkofel	2	In situ	●	●	●	Cartellieri (1935)
1956	Mt. Patscherkofel	1	Lab	●	n d	n d	Pisek and Winkler (1958)
1966	Mt. Patscherkofel	2	In situ	●	●	●	Koch et al. (1968)
1979	Mt. Patscherkofel	1	In situ	●	n d	n d	Havranek (1981)
1996	Mt. Patscherkofel	1	In situ	n d	●	n d	Wieser et al. (2000)
2002	Mt. Patscherkofel	2	In situ	●	n d	n d	Wieser (2004)
2007	Mt. Patscherkofel	3	In situ	●	n d	n d	Wieser et al. (2010)
2012	Haggen	6	In situ	●	●	●	Wieser et al. (2015)

Fig. 1. Temporal variation in (**a**) mean annual air temperature (T_{air}), (**b**) pressure corrected vapour pressure deficit (Δw), and (**c**) total annual precipitation (P) during the period 1925 throughout 2013 at treeline on Mt Patscherkofel 1950 m a.s.l. See Table 2 for regression information.

BAI, $\delta^{13}C$, and iWUE. These statistical analyses were based on mean annual T_{air}, annual mean Δw, and total P. Regression and correlation analysis were performed using the SPSS 16 software package (SPSS. Inc. Chicago, USA), and a probability level of $P < 0.05$ was considered as statistically significant. In order to avoid the risk of removing any environmental signal or trend captured by our tree-ring series, we did not remove any age related trend from our tree-ring chronologies by conventional de-trending procedures (c.f. also Brito et al., 2016).

3. Results

3.1. Inter-annual trends in environmental conditions

Linear regression analysis reveal a significant increase in mean annual T_{air} during the period 1925–2013, mainly due to the warming trend since 1982, while a considerable trend in T_{air} was absent from 1925 to 1981 (Fig. 1a, Table 2). On an annual scale, increasing T_{air}
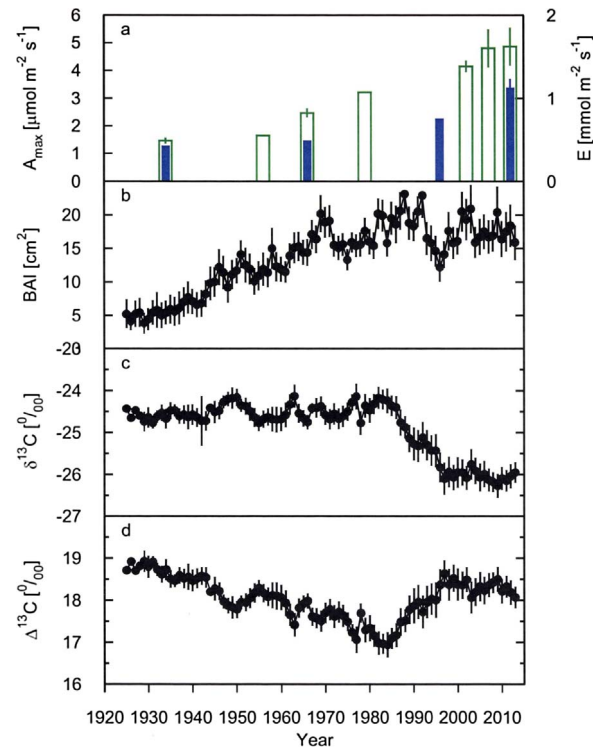

Fig. 2. Temporal variation in (**a**) net photosynthetic capacity at ambient CO_2 (A_{max}; green bars), transpiration (E; blue bars), (**b**) basal area increment (BAI), (**c**) tree-ring $\delta^{13}C$ ($\delta^{13}C$), and (**d**) carbon discrimination ($\Delta^{13}C$), chronologies of *P. cembra* between 1925 and 2013. See Table 2 for regression information. A_{max}, and E values are the mean $\pm$ SE of 1–6 trees (c.f. also Table 1), BAI values are the mean $\pm$ SE of 10 trees, while $\delta^{13}C$ and $\Delta^{13}C$ values are the mean $\pm$ SE of 6 trees.

coupled with a decline in RH (data not shown) caused an overall significant increase in Δw throughout the study period, although Δw declined significantly since 1998 (Fig. 1b, Table 2). Precipitation by contrast, revealed no long-term trend (Fig. 1c, Table 2).

3.2. Leaf level gas exchange

Over the entire study period A_{max} of mature field grown *P. cembra* measured at treeline increased significantly ($r^2 = 0.96$, $P < 0.001$) from 1.5 µmol m^{-2} s^{-1} in 1934–4.9 $\pm$ 0.7 µmol m^{-2} s^{-1} in 2012 (Fig. 2a). The increase in A_{max} was accompanied by a rise in E from 0.42 mmol m^{-2} s^{-1} in 1934–1.12 $\pm$ 0.11 mmol m^{-2} s^{-1} in 2012

Table 2

Characteristics of air temperature (T_{air}), pressure corrected vapour pressure deficit (Δw), precipitation (P), maximum net CO_2 uptake rate at ambient CO_2 (A_{max}), transpiration (E), basal area increment (BAI) stable carbon isotope ratio ($\delta^{13}C$), C discrimination ($\Delta^{13}C$), internal CO_2 concentration (C_i), and intrinsic water-use efficiency (iWUE) in *P .cembra* during the periods 1925–2013, 1925–1981, 1982–1997, and 1998–2013. Significant changes from 1925 to 2013, 1925–1981, 1982–1997, and 1998–2013 are in bold and italics. Significant levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Between period differences in average ($\pm$ SD) at $P < 0.05$ are marked with different letters. Change values per decade were calculated as the slope of the corresponding least-squares linear regressions multiplied by 10.

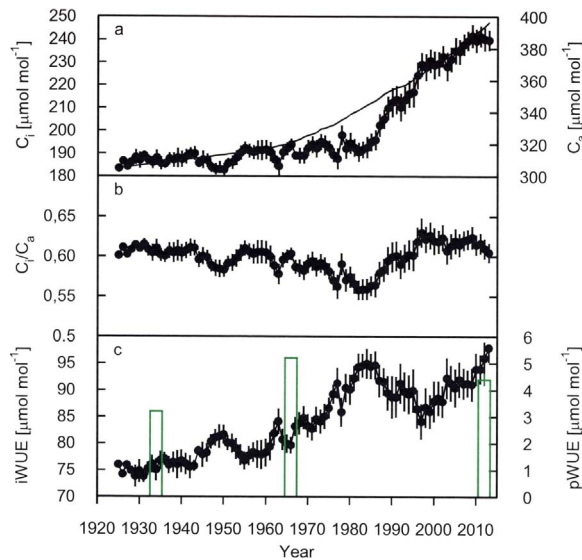|	1925–2013		1925–1981		1982–1997		1998–2013	
	Change	Average ($\pm$ SD)	Change	Average ($\pm$ SD)	Change	Average ($\pm$ SD)	change	Average ($\pm$ SD)
T_{air} [°C]	***0.2****	2.1 (0.9)	0	1.6(0.7)[a]	0.4	2.7 (0.8)[b]	0.0	3.1 (0.6)[c]
Δw [Pa kPa^{-1}]	***0.06****	1.97 (0.28) [a]	0.01	1.4 (0.4)[a]	−0.06	2.1 (0.3)[b]	***−0.1****	1.7 (0.3)[c]
P [mm]	7	880 (132)	21	878 (135)[a]	−40	857 (88)[a]	−23	911 (148)[a]
A_{max} [µmol m^{-2} s^{-1}]	***0.48****	3.25 (1.43)	0.41	2.19 (0.82)[a]			0.67	4.62 (0.36)[b]
E [mmol m^{-2} s^{-1}]	0.08	0.69 (0.27)						
BAI [cm^2]	***1.6****	13.6 (5.1)[a]	***2.5****	11.2 (4.4)[b]	***−3.7****	18.2 (3.1)[c]	−0.3	17.6 (1.7)[c]
$\delta^{13}C$ [‰]	***−0.18****	−24.7 (1.8)	0.02	−24.5 (0.2)[a]	***−1.2****	−25.0 (0.6)[b]	−0.1	−26.0 (0.1)[c]
$\Delta^{13}C$ [‰]	0.0	17.9 (0.4)	***−0.1****	17.9 (0.3)[a]	***1.0****	17.7 (0.5)[b]	***−0.1****	18.3 (0.1)[c]
C_i [µmol mol^{-1}]	***6****	201 (19)	***1****	189 (3)[a]	***24****	208 (12)[b]	***10****	235 (5)[c]
C_i/C_a	0	0.60 (0.02)	0	0.60 (0.01)[a]	***0.04****	0.59 (0.02)[b]	0.0*	0.62 (0.00)
iWUE [µmol mol^{-1}]	***2.4****	84 (7)	***2.5****	80.0 (4.7)[a]	***−6.0****	90.9 (3.2)[b]	***6.3****	91.1 (3.2)[b]

Fig. 3. Temporal variation in (**a**) intercellular CO_2 concentration (C_i, solid circles), ambient atmospheric CO_2 concentration (C_a, thin solid line), (**b**) C_i/C_a, and (**c**) intrinsic water-use efficiency (iWUE), and instantaneous WUE of photosynthesis (pWUE) of *P. cembra* between 1925 and 2013. See Table 2 for regression information. C_i, C_i/C_a, and iWUE values are the mean ± SE of 6 trees, while PWUE values are values are the mean ± SE of 1–6 trees.

(Fig. 2a), which however was not significant ($r^2 = 0.83$, $P = 0.086$) along with an increase in the atmospheric CO_2 concentration (from 310 to 390 ppm). pWUE remained unchanged between 1934 and 2012 and averaged 4.3 ± 0.7 μmol mol^{-1} (Fig. 3c).

3.3. Long-term variabilities in BAI, $\delta^{13}C$, $\Delta^{13}C$, C_i, C_i/C_a and iWUE

The study trees were even aged and the expressed population signal (EPS) was 0.85, suggesting adequate replications and a strong common climate signal in our treeline chronology. We detected a significant increase in BAI over the entire study period, especially from 1925 to 1981. BAI declined significantly from 1982 to 1997, and did not change considerably after 1998 (Fig. 2b, Table 2). Tree-ring $\delta^{13}C$ values stayed constant until 1981, declined significantly from 1982 to 1997, and did not change noticeably after 1998 (Fig. 2c, Table 2). Accordingly, carbon isotope discrimination ($\Delta^{13}C$ after the tree ring specific $\delta^{13}C$ correction for the progressive decline in atmospheric $\delta^{13}C$) declined until 1981 and increased from 1982 to 1997 (Fig. 2d, Table 2).

Paralleling atmospheric CO_2 enrichment (Fig. 3a) tree-ring derived C_i increased significantly from 183 μmol mol^{-1} in 1925–240 μmol mol^{-1} in 2013 (Fig. 3a, Table 2). The ratio of C_i/C_a revealed no considerable overall trend from 1925 through 2013, although there was a slight but significant increase from 1982 to 1997 (Fig. 3b, Table 2). *Pinus cembra* significantly increased its iWUE from 1925 to 1981 and from 1993 to 2013, while the opposite trend was found for the period 1982–1997 (Fig. 3c, Table 2).

3.4. A_{max}, BAI, $\delta^{13}C$, and iWUE in response to climate

Pearsoń́s correlations revealed strong links between environmental parameters and A_{max}, BAI, $\delta^{13}C$, and iWUE, respectively (Table 3). At an annual scale A_{max} was positively correlated with C_a and T_{air} (Table 3). BAI was significantly positively correlated with C_a, Δw, and T_{air} over the entire study period (1925–2013) (Table 3). The correlations of C_a and Δw with BAI were also positive and significant for the period 1925–1981, which was not the case for T_{air}. For the period 1982–1997 the correlation between C_a and BAI was significantly negatively. $\delta^{13}C$ correlated significantly negatively with C_a, Δw, and T_{air} for the entire study period 1925–2013 (Table 3). For the period 1925–1981, the

Table 3
Pearsoń́s correlation coefficients between maximum net CO_2 uptake rate at ambient CO_2 (A_{max}), basal area increment (BAI) stable carbon isotope ratio ($\delta^{13}C$), and intrinsic water−use efficiency (iWUE) of *Pinus cembra* and environmental data (atmospheric CO_2 concentration (C_a), pressure corrected vapour pressure deficit (Δw), and mean annual air temperature (T_{air}) calculated for different periods. Significant values are in bold and italics. Significant levels: * $P < 0.05$, ** $P < 0.01$, $P < ***$.

Parameter	Period	C_a	Δw	T_{air}
A_{max}	1934–2012	***0.984***	0.404	*0.768*
BAI	1925–2013	***0.708***	***0.507***	***0.496***
	1925–1981	***0.839***	*0.269*	0.097
	1982–1997	*−0.535*	0.026	0.252
	1998–2013	−0.111	0.342	0.176
$\delta^{13}C$	1925–2013	***−0.851***	***−0.366***	***−0.560***
	1925–1981	0.201	**0.370**	0.258
	1982–1997	***−0.978***	0.021	−0.319
	1998–2013	−0.399	*0.515*	0.356
iWUE	1925–2013	***0.855***	***0.569***	***0.561***
	1925–1981	***0.944***	***0.450***	0.163
	1982–1997	***−0.911***	−0.093	−0.369
	1998–2013	***0.925***	−0.387	0.122

correlation between Δw and $\delta^{13}C$ was significantly positively, while the correlation between C_a and $\delta^{13}C$ was significantly negatively for the period 1982–1997 (Table 3). C_a was also positively correlated with iWUE during the periods 1925–1981 and 1998–2013, while the effect of C_a on BAI during the period 1982–1997 was clearly in opposite direction (Table 3). Δw and BAI also displayed a positive correlation over the whole study period and from 1925 to 1981 (Table 3).

We also found correlations of A_{max} with tree growth and tree-ring $\delta^{13}C$ (Fig. 4). BAI was significantly positively correlated with increasing A_{max} ($P = 0.02$; Fig. 4a), whereas, tree-ring $\delta^{13}C$ was significantly negatively correlated with A_{max} ($P = 0.01$; Fig. 4b). Furthermore, A_{max} tended to increase with iWUE (data not shown), and hence also with tree-ring derived C_i, similar to a classical A/C_i curve (Fig. 5).

4. Discussion

The breakpoint of our segmented analysis on the long term patterns of BAI, $\delta^{13}C$ in tree-rings, $\Delta^{13}C$ and iWUE in 1981 (Fig. 2b, c, d; Fig. 3c) is in agreement with the observed rise (starting point 1982) in regional T_{air} (Fig. 1a) and a loss in the temperature response of radial growth (Table 3; Oberhuber et al., 2008), strongly suggesting a different response to rising C_a (Saurer et al., 2004) before and after 1981. After 1981 BAI remained largely stable, indicating that our 125-year-old study trees had matured after ≈ 95 years of growth, and subsequently also displayed a diminishing sensitivity to rising C_a. No trend in BAI is a common feature in mature trees (Peters et al., 2015), contrary to
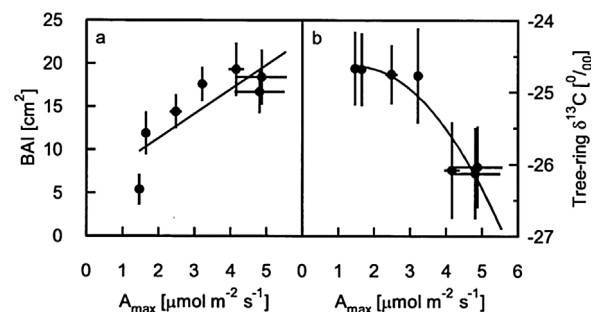

Fig. 4. Total needle surface area based maximum net CO_2 uptake rate at ambient CO_2 (A_{max}) obtained from mature field grown *P. cembra* trees carried out at treeline between 1934 and 2012 versus (**a**) basal area increment (BAI) and (**b**) tree-ring $\delta^{13}C$. Points were fitted by linear regression analysis: BAI: $y = 2.83x + 5.67$, $r^2 = 0.69$, $P = 0.02$; tree-ring $\delta^{13}C$: $y = (−0.14x + 0.41)x − 24.97$, $r^2 = 0.89$, $P = 0.01$. BAI values are the mean ± SE of 10 trees, tree-ring $\delta^{13}C$ values are the mean ± SE of 6 trees, and A_{max}, values are the mean ± SE of 1–6 trees.
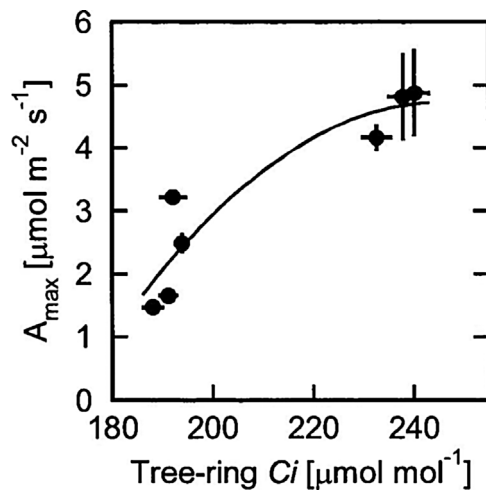
Fig. 5. Relationship between total needle surface area based maximum net CO_2 uptake rate at ambient CO_2 (A_{max}) obtained from mature field grown *P. cembra* trees carried out at treeline between 1934 and 2012 and tree-ring derived intercellular CO_2 partial pressure (C_i). Points were fit by polynomial regression analysis: $y = -0.009 \times^2 + 0.42 \, x - 47.3$, $r^2 = 0.87$, $P < 0.005$. Tree-ring $\delta^{13}C$ values are the mean ± SE of 6 trees, and A_{max}, values are the mean ± SE of 1–6 trees.

diameter growth that often shows decreases with increasing tree size (Martinez-Vilalta et al., 2008; Silva et al., 2010), as also observed in mature *P. cembra* trees at our study site (Oberhuber et al., 2008). By contrast, 70-year old *P. cembra*, *P. abies*, and *L. decidua* trees nearby showed a significant increase in BAI during the period 1975–2010, being significantly lower in *P. cembra* (1.5 cm² per decade) than in *P. abies* (2.2 cm² per decade), and *L. decidua* (2.7 cm² per decade) (Wieser et al., 2016). Nevertheless, it has to be noted that BAI may not effectively disentangle age/size from long-term growth trends, if a species shows a continually increasing trend in basal area growth over its life (Peters et al., 2015).

Changes in $\delta^{13}C$ in tree-rings, $\Delta^{13}C$, and iWUE from 1925 to 1981 were closely related to the predicted changes in a scenario with constant C_i, which is related to the strong response to increasing C_a (Saurer et al., 2004). Under this scenario, iWUE exceeds the expected value resulting from an active response to increased C_a, suggesting that stomatal narrowing at treeline may be reinforced by other factors (Andreu-Hayles et al., 2011; Wieser et al., 2016; Wu et al., 2015). After 1981 the effect of rising C_a on iWUE changed. From 1982–1997 C_i changes proportionally with C_a suggesting that the plants change from an active (keeping C_i constant) to a passive response (keeping C_a-C_i constant) (McCarroll et al., 2009). This also represents a relatively weak stomatal response, as iWUE decreases. No change in iWUE (i.e., homeostasis) since the 1980s has been reported for *L. decidua* at treeline in the Swiss Alps (Churakova et al., 2016) and for *Picea schrenkiana* at treeline in the western Tianshan Mountains of China (Wu et al., 2015). From 1997 onwards $\delta^{13}C$ in tree-rings stays constant, because of constant C_a/C_i ratio along with a constant $\Delta^{13}C$. Accordingly, iWUE increases again, reflecting a proportional regulation of A and g_w presumably in response to climate warming. Conversely, the observed increase in iWUE before 1981 (c.f. also Waterhouse et al., 2004) may be explained by a C_a-induced increase in A_{max}, combined with no change in g_w and water loss, given that T_{air} remained unchanged while the evaporative demand (in terms of Δw), tended to increase (Fig. 1a, b; Table 2).

Long term variations in $\delta^{13}C$ and C_i were also partly a result of varying carbon demand for CO_2 by photosynthesis (Farquhar et al., 1998) due to a temperature driven increase of carbon investment into growth, since temperature was no longer growth limiting since 1981, enhancing the carbon demand. This is supported by a positive correlation between BAI and A_{max} (Fig. 4a) and a negative correlation between tree-ring $\delta^{13}C$ and A_{max} (Fig. 4b). Moreover, A_{max} in *P. cembra*

does not vary significantly between the upper sun-and the lower shade-crown as exemplified at our treeline site by Pisek and Winkler (1958) and Wieser (2004) in 1956 and 2002, respectively. The corresponding A_{max} values for sun and shade needles were 1.65 and 1.74 μmol m^{-2} s^{-1} (Pisek and Winkler, 1958) in 1956, and 4.16 ± 0.19 and 4.67 ± 0.11 μmol m^{-2} s^{-1} in 2002 (Wieser, 2004), respectively. The similarity in the magnitude of A_{max} and total growing season net CO_2 uptake in the upper sun-and the lower shade-crown of mature *P. cembra* trees at treeline (Wieser et al., 2005) may be due to an open stand structure, resulting in low intra-canopy light gradients within entire crowns as well as a higher coupling of gas exchange to the atmosphere relative to closed-canopy conditions at lower elevation sites (Wieser and Tausz, 2007).

Temporal changes in tree-ring derived iWUE were also mirrored in leave level gas exchange. At treeline in the Central Austrian Alps A_{max} of sun-exposed twigs from the upper canopy of mature field grown *P. cembra* increased significantly between 1934 and 2012 (Fig. 2a). Although gas exchange measurements varied widely depending on instrumentation and methodological procedures (Table 1) our observed increase of 0.48 μmol m^{-2} s^{-1} per decade in A_{max} between 1934 and 2012 did not differ statistically significant compared to the increase of the 0.52 μmol m^{-2} s^{-1} per decade obtained from 1966 to 2012 (t value 0.36, probability 0.73; two slope comparison test according to Zar, 1999) when A_{max} was determined in situ by means of thermoelectrically climate-controlled cuvettes and a portable exchange system (Table 2). At our study site on Mt. Patscherkofel, increasing A_{max} during the last decades has also been reported for adult *P. abies* and *L. decidua* trees. A_{max} of *P. abies* increased from 0.9 μmol m^{-2} s^{-1} in 1956 (Pisek and Winkler, 1958) to 3.3 and 3.1 μmol m^{-2} s^{-1} in 1986 and 1987, respectively (Havranek et al., 1989). Likewise A_{max} of *L. decidua* increased from 3.3 μmol m^{-2} s^{-1} in 1980 (Benecke et al., 1981) to 5.6 ± 0.9 μmol m^{-2} s^{-1} in 1993 (Volgger, 1995).

A potential explanation for the observed tripling of A_{max} from 1934 till now may be that under equal CO_2 partial pressure (after pressure corrections according to Von Caemmmerer and Farquhar (1981) trees at treeline display considerably higher in situ net CO_2 uptake rates as compared to their relatives at a low elevation sites; as shown for *P. abies* in the central European Alps (Wieser, 2007) and for *Espeletia schulztii* along an altitudinal gradient in the Venezuelan Andes (Rada et al., 1998). Higher net CO_2 uptake rates indicate a higher efficiency of carbon uptake per unit leaf area in trees at treeline. Equal A_{max} in trees at treeline (Benecke et al., 1981; Wieser, 2007), and a higher carboxylation efficiency per unit leaf area might be due to elevation related differences in needle morphology. Conifers at treeline generally produce less needle area per unit of needle dry mass (Benecke et al., 1981; Birmann and Körner, 2009; Hultine and Marshall, 2000; Richardson et al., 2001). For example, on a total needle surface area basis, the specific leaf area of current-year *P. abies* needles has been shown to decrease from 186 to 104 cm² per g dry weight between 1000 and 1950 m a.s.l. (Wieser 2007). Needles of trees at high elevation sites also have similar or higher nitrogen (N) concentrations per unit dry mass when compared to lower elevation sites (Birmann and Körner, 2009; Körner, 1989; Richardson et al., 2001; Sparks and Ehleringer, 1997), indicating no nitrogen depletion at treeline. Moreover, treeline sites have a lower atmospheric pressure, probably increasing the effect of rising C_a on net CO_2 uptake rate more than at lower elevation sites (Hultine and Marshall, 2000). Greater diffusivity (lower specific leaf area ≈ thicker needles) and higher N suggest higher net CO_2 uptake rates in tree growing at timberline, as shown in CO_2 response curves. For instance, an increase in ambient CO_2 concentration from 310 μmol mol^{-1} to 390 μmol mol^{-1}; which matched the observed increase in C_a from 1934 till now, resulted in an increase in A_{max} of 30–100% as derived from A/C_i curves obtained for conifers at treeline sites in the Swiss (Streit et al., 2014) and Austrian Alps (Wieser, unpublished), which is considerable above the value (20%) obtained for conifers at low elevation sites (Wieser and Tausz, 2007). A greater

demand for CO_2 in treeline trees is also reflected in an elevational increase in $\delta^{13}C$ of needle dry matter or cellulose (Hultine and Marshall, 2000; Körner et al., 1988, 1991).

The observed increase in A_{max} of *P. cembra* can be ascribed to both the observed increase in T_{air} and C_a. Additionally, during the period 1956–2007 temperature optimum of A_{max} for *P. cembra* increased from 12.5 °C (Pisek et al., 1969) to 17.1 °C (Wieser et al., 2010). Climate warming is anticipated to increase evapotranspiration (Saxe et al., 2001). Our observed rise in A_{max} was accompanied by an increase in E, which however, was not statistically significant (Fig. 2a, Table 2), and constant pWUE (Fig. 3c) suggests that g_w had increased during the past 89 years. Similarly, ecosystem warming substantially increased transpiration in *P. cembra* at treeline in the Central Austrian Alps, which can be attributed to a warming-induced root production, a reduction in viscosity of water and/or perhaps an increase also in root hydraulic conductivity so that leaf-level pWUE remained unaffected (Wieser et al., 2015). Thus, it seems that in environments under non-limiting water availability, as it is the case at treeline in the Central Austrian Alps (Wieser, 2012), increasing temperature counteracts the diminishing effect of rising C_a on g_w (Marchin et al., 2016; Saurer et al., 2014).

5. Conclusions

The use of combined analysis of long-term leaf-level gas exchange data, radial growth, and carbon isotope analysis provided new insights into long-term trends of tree-ring derived iWUE. *P. cembra* at treeline responds to the increasing C_a and T_{air} with a parallel increase in A_{max} and tree-ring-derived C_i. We found an increase in BAI and iWUE before 1981. After 1981, the sensitivity of iWUE to increasing C_a diminished, and was accompanied by fairly stable BAI. Stable iWUE suggests a stable ratio between carbon gain and leaf conductance for water vapour as also indicated by foliar gas-exchange data. Our observed stability in tree growth over the past 30-years suggests that thereafter our study-trees are much less limited by CO_2 and temperature. Therefore, our observed (distinctive) back-and-forth in iWUE after 1981 when BAI remained fairly stable, may probably be related to a decoupling of BAI (tree growth) from increasing C_a and A_{max}, combined with an age-related change in carbon allocation and accumulation patterns. Factors directly controlling the dynamics of carbon allocation patterns in individual trees remain largely unknown, and include genetic, biochemical, physiological source sink regulations (Gower et al., 1995), besides competition among trees (Nilsson and Albrekstson, 1993).

Acknowledgements

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